Prevalence and Antibiotic Susceptibility of *Salmonella* Strains Isolated from Viscera of Quail (*Coturnix coturnix japonica*) Breeds in Bingerville Area, Côte d'Ivoire

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Abstract

The control of food products contamination by *Salmonella* has become essential for the consumer to ensure the safety of these foods. Poultry meat and eggs play an important role as infection risk factors related to *Salmonella* strains resistant to antibiotics. Quail breeding, which is becoming to be appreciated by the Ivoirian population after chicken meat, may contain *Salmonella* pathogenic strains. Thus, a preliminary survey in two areas of high quail production (ANNA and SANTAI) in the city of Bingerville (Côte d'Ivoire), allowed collecting 60 viscera of quail for microbiological analysis. The microbiological analysis revealed 25 strains of *Salmonella* isolated from 11 viscera, resulting in a prevalence rate of 18.33 %. These strains also showed high resistance to Tetracycline (100 %), a relatively average resistance to Nalidixic acid (24 %) and low resistance to Ampicillin (8 %), Ticarcillin (8 %) and Cotrimoxazole (8 %). The presence of antibiotic-resistant strains of *Salmonella* in quail viscera could constitute a public health problem.

Keywords: Quail, *Salmonella*, Antibiotic resistance, Food safety, Bingerville (Côte d'Ivoire)

Introduction

The poor microbiological quality of food is responsible for foodborne illnesses and causes considerable damage in the world. This makes Food Security a major challenge for governments, consumers and producers. The pathogenic microorganisms commonly found in foods are

Campylobacter, Salmonella, Escherichia coli, Staphylococcus and *Clostridium.* The genus *Salmonella* is the main cause of foodborne gastroenteritis in humans (Gordana *et al.,* 2012). Indeed, *Salmonella* infections, also known as salmonellosis, account for 22 millions of disease cases per year in the world. This proportion makes infections due to *Salmonella* a threat to public health (Goburn *et al.,* 2007).

The emergence of *Salmonella* resistant to antibiotics has become an epidemiological problem. The development of antibiotic-resistant against the bacteria is also another public health problem in the world. *Salmonella* antibiotic-resistance continues to increase as a result of excessive use of antibiotics in animal husbandry (WHO, 2010).

In recent decades, *Salmonella* resistant to antibiotics have been recognized as a threat to public health in most developing countries. Thus the need to control *Salmonella* contamination of food products is necessary.

Antibiotic resistant *Salmonella* strains have been isolated from chickens in several countries (Bonny *et al.,* 2011; Bonny *et al.,* 2014; Karou *et al.,* 2013; Ouattara *et al.,* 2013)**,** turkeys (Karraouan *et al.,* 2010) and ducks (Adzitey *et al.,* 2011). Quail meat is becoming more and more appreciated by consumers because it contains a good proportion of protein just like the other products, an appreciable amount of trace elements, and a varied fat content. In addition, this meat is accessible to all budgets.

This study was undertaken in order to establish a database on antibiotic resistance of *Salmonella* strains isolated in poultry in Côte d'Ivoire, by evaluating the level of antibiotic of *Salmonella* isolated from farmed quail in Côte d'Ivoire.

Material and methods

Sampling

This study was conducted in two (2) villages (ANNA and SANTAI) in the area of Bingerville, in Abidjan District (**Figure 1**). A non-random sampling method allowed to retain two (2) quail farms with more than 2,000 quail heads, which supplied poultry and eggs to restorators and poultry markets. In each farm, Thirty (30) quail (**Photography 1**) were bought, slaughtered and the viscera (**Photography 2**) was collected. At each site, 10 viscera were collected per week. Each viscera was placed in a sterile stomacher bag and placed in a cooler containing cold accumulator and, then transported to the laboratory for analyses.

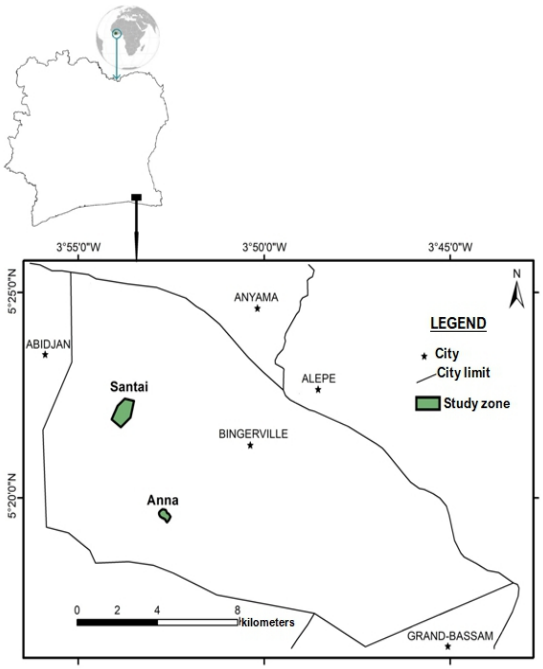
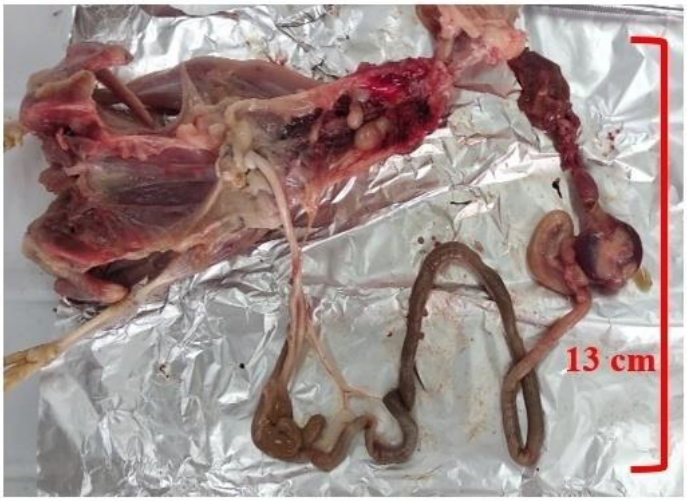


Figure 1: *Location of the study areas*

Photography 1: *Quail breeds (Coturnix coturnix japonica)*



Photography 2: *Quail viscera*

Bacteriology test for the isolation of *Salmonella spp.*

All strains were isolated according to the protocol described by standard ISO 6579 (ISO-6579-1, 2017) in 4 steps. The primary enriching stage often (10) grams of crushed quail viscera in 90 mL of BPW (Buffered Peptone Water) (BIORAD, France); a selective enrichment stage of 0.1 mL and 1 mL of the pre-enriched broth in respectively 10 mL of Rappaport Vassilliadis broth (BIO-RAD) and Müller-Kauffman Tetrationate broth (BIO-RAD, France); the isolation of presumptive strains onto two selective agar plates namely Hecktoen and XLD (Xylose-Lysine-Desoxycholate) media (BIORAD, France).

Salmonella presomptive colonies obtained were tested for catalase and oxidase test and Gram control. Finally, the isolates were identified on the reduced rack of LEMINOR constituted of four culture media (Kligler-Hajna/ Mannitol-Mobility/Urea indol/Simmons Citrate) to determine its biochemical characteristics.

Analysis of suspicious strains of Salmonella by the MALDITOF (*matrix assisted laser desorption ionisation time-of-flight*)

For the confirmation of the genus *Salmonella* spp, 1µl of each colony of *Salmonella* presumptive strains was removed and then deposited on target wells of a slide (DS) (BIOMERIEUX). Subsequently, 1µL of matrix CHCA (α-cyano-4hydroxycinnamic acid) was added. After drying at room temperature, the blade was placed on a blade holder (BIOMERIEUX), and the whole was introduced into the Vitek MS (BIOMERIEUX) for analysis. *Escherichia coli* ATCC 8739 reference strain contained in one of the wells of the DS blade (BIOMERIEUX) is used as a standard.

Antibiotic susceptibility testing

After confirming biochemical tests, *Salmonella* strains were tested for their resistance to some antibiotic used in both veterinary and human medicine. These tests were carried out by diffusion in an agar medium, according to CLSI standard (Clinical Laboratory Standard Institute) on Müller-Hinton agar (BioRad, France) (CLSI, 2005). The antibiotic discs (BioRad, France) used to demonstrate the resistance profile of isolated *Salmonella* strains were: amoxicillin (AMX, 25µg), Cefalotin (CF, 30 µg), Cefoxitin (FOX, 30 µg), Cefotaxime (CTX, 30 µg), Ticarcillin (TIC, 75 µg), Chloramphenicol (C, 30 µg), Gentamicin (GM, 10µg), Nalidixic Acid (Nal, 10 µg), Ciprofloxacin (Cip, 10 µg), Cotrimoxazole (SXT, 10/20 µg), Tetracycline (TE, 10 µg), Tobramycin (TM, 10 µg). The reference strains of *Salmonella* named ATCC 14028, was used to validate the antibiogram test.

Results

Bacteriology tests for the isolation of Salmonella spp.

Microbiological analysis of the gastrointestinal tract of the quail marketed in the town of Bingerville allowed to determine their microbiological status compared to *Salmonella*. Thus, of the 60 viscera of quails analyzed, 11 viscera were contaminated. Of these 11 viscera contaminated, 25 presumptive strains of *Salmonella* were isolated, a rate of 18.33 % and an isolation rate of 41.66 %. The 25 presumptive isolates of *Salmonella* isolated had the following biochemical characteristics: Gram negative (**Gram**$^-$); presence of catalase (**Catalase** $^+$); absence of an oxidase (**Oxidase**$^-$); Aero-Anaerobic Optional (**AAF**); ferment glucose (**Glu**$^+$); do not ferment lactose (**lactose**$^-$); ferment mannitol (**mannitol**$^+$); production of hydrogen sulphide (**H_2S** $^+$); gas production (**gas** $^+$); use of citrate as a carbon source (**citrate** $^+$); lacking urease (**urea**$^-$); lacking tryptophanase (**indole**$^-$); peritrich mobility (**mobility** $^+$).

Analysis of suspicious strains of *Salmonella* by the MALDITOF (*matrix assisted laser desorption ionisation time-of-flight*)

Confirmation of the genus of these presumptive strains was carried out on Vitek MS of MALDITOF. This method made it possible to obtain an identification percentage of 99.9 % of these bacterial strains. So, all the bacterial strains belong to the genus *Salmonella* spp.

Antibiotic susceptibility testing

The 25 strains of *Salmonella* spp. were analyzed for their resistance to the 12 antibiotics. This study allowed to classified, according to the CLSI standards in: resistant or sensitive. *Salmonella* strains isolated from quail viscera showed low resistance to Amoxicillin, Ticarcillin and Cotrimoxazole

(8 %), a relatively average resistance to Nalidixic Acid (24 %). However, all isolated strains showed resistance to tetracycline (100 %). For Cephalotin, Cefoxitin, Cefotaxime, Gentamycin, Tobramycin, Chloramphenicol, and Ciprofoxacin, no resistance was observed (0 %) (**Figure 2**).

The overall profile of antibiotic resistance of *Salmonella* spp. isolated from quail viscera is presented in **table 1**. Analysis of the resistance profiles revealed two levels of antibiotic resistance (**Table 2**). The first level of resistance refers to 15 isolated strains with only one type of profile (**TE**), involving only the Cyclin family. The second level of resistance concerns the resistance of isolated strains (10 strains) to two (2) families with different combinations including Cyclins, Quinolones, Sulfamides and β-lactams. The different resistance profiles observed at this level are: **SXT-TE** (2 strains); **TE-NA** (6 strains); **TE-TIC-AMX** (2 strains). However no strain of *Salmonella* isolated from quail viscera in this study was found to be multidrug-resistant.

Table 1: *Global Antibiotic Resistance (ATB) Profiles of Salmonella spp. Strains Isolated from Quail Viscera*

ATB / Strains	AMX	TIC	CEF	FOX	CTX	GEN	TM	CHL	SXT	CIP	AN	TE	Resistance Profiles
S7H1C	S	S	S	S	S	S	S	S	**R**	S	S	**R**	SXT-TE
S7H1A	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S7X1C	S	S	S	S	S	S	S	S	S	S	**R**	**R**	TE-NA
S7H1B	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S7X1A	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S7X1B	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S4H1A	S	S	S	S	S	S	S	S	**R**	S	S	**R**	SXT-TE
S17H2C	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S17H2B	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S22H3D	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S22H3A	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S22H3C	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S23H3A	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S26H3B	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S26H3A	**R**	**R**	S	S	S	S	S	S	S	S	S	**R**	TE-TIC-AMX
S26H3C	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S29H3C	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S29H3B	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
S30H3C	**R**	**R**	S	S	S	S	S	S	S	S	S	**R**	TE-TIC-AMX
A7H1B	S	S	S	S	S	S	S	S	S	S	**R**	**R**	TE-NA
A9H1A	S	S	S	S	S	S	S	S	S	S	S	**R**	TE
A9S1B	S	S	S	S	S	S	S	S	S	S	**R**	**R**	TE-NA
A11H2C	S	S	S	S	S	S	S	S	S	S	**R**	**R**	TE-NA
A11S2A	S	S	S	S	S	S	S	S	S	S	**R**	**R**	TE-NA
A11H2B	S	S	S	S	S	S	S	S	S	S	**R**	**R**	TE-NA

AMX:Amoxicillin; CF: Cefalotin; FOX: Cefoxitin; CTX: Cefotaxim; TIC: Ticarcillin; C: Chloramphenicol; GM: Gentamicyn; Nal: Nalidixic Acide; Cip: Ciprofloxacin; SXT:Cotrimoxazole; TE:Tetracycline; TM:Tobramycin.

Table 2: *Levels of resistance to antibiotic families of Salmonella spp strains isolated from Quail Viscera*

Levels	Number of antibiotic	Resistance Profile	Number of strains	Familly involved
Level 1	1	TE	15	Cycline
		SXT-TE	2	
	2	TE-NA	6	Sulfamide, Cyclin,
Level 2	3	TE-TIC-AMX	2	Quinolone, β-lactamins

AMX: Amoxicillin; **NA**: Nalidixic Acid; **TE** : Tectracyclin; **TIC**: Ticarcillin; **SXT**: Cotrimoxazole.

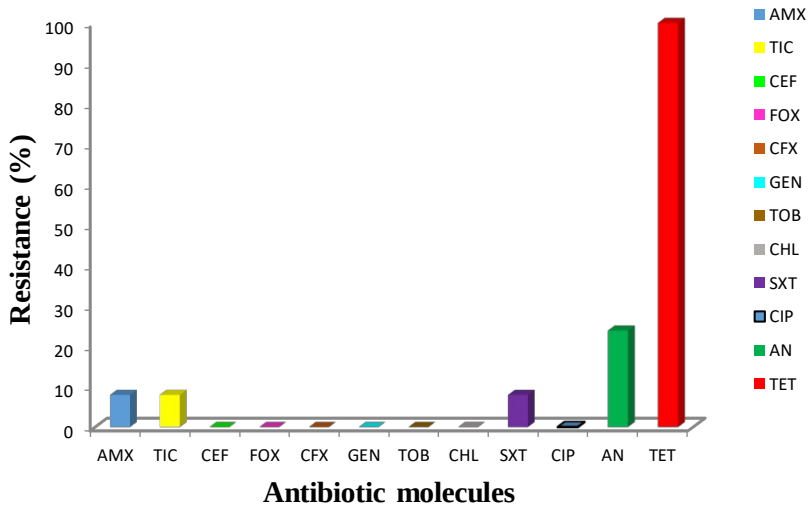


Figure 2 *: Levels of resistance to antibiotic of Salmonella spp strains isolated from Quail Viscera*

Discussion

The presence of *Salmonella* strains in quail viscera, revealed a contamination of these viscera with a carriage rate estimated at 18.33 %. That confirms the hypothesis that *Salmonella* strains are present in poultry. Indeed, previous studies on other types of poultry also revealed the presence of *Salmonella* strains in the raw gizzard of broilers in Turkey (18.6 %) (Carli *et al.*, 2001), at the level of raw turkey minced meat in Morocco (20.31 %) (Karraouan *et al.*, 2010), Chicken carcasses in Tchad (65.85 %) (Abba *et al.*, 2017), in the intestines of chicken at the stage of slaughter in Côte d'Ivoire (56 %) (Traoré, 2003) and on raw chicken gizzard samples (61.87 %) in Côte d'ivoire (Bonny, 2015), the weavers faeces in Cameroon (78.46 %) (Piebeng *et al.*, 2014)**,** on avian products (chicken carcass, turkey carcass) in the Czech

Republic (16.6 %) (Myskovâ and Karpîskovâ, 2017), on the chicken livers (35 %), gut (30 %), lung (10 %) and kidney (15 %) in Pakistan (Rafiullah *et al.*, 2018). Also, Carli *et al.* (2001) reports that the rate of contamination at the level of poultry farms by *Salmonella* was estimated at 41.3 % in Turkey, while in Sénégal, Cardinale *et al.* (2004) reported an estimated carry rate of 28.6 %.

The presence of *Salmonella* in the quail viscera could facilitate cross-contamination during laughter, in case of defective hygienic practices during the production of poultry products (Bonny *et al.*, 2018). In view of these results, it is worth noting that the exact origin of *Salmonella* contamination of poultry is still unknow, but it is thought to be related to their diet, drinking water, environmental conditions of cleaning and maintenance.

The *Salmonella* strains isolated were found to belong to the genus *Salmonella* by using the technical of MALDI-TOF (99.99 %). They were also tested for their susceptibility to 12 antibiotics commonly used in veterinary and human medicine.

The results show that all the 25 isolates of *Salmonella* exhibit total resistance to tetracycline (100 % TE). The high level of resistance obtained at the tetracycline level in this study is similar to the rate obtained in chicken samples in Iran by (Fallah *et al.*, 2013). A high level of tetracycline (73 %) was also obtained by Bonny *et al.* (2014), on the raw chicken gizzards in Côte d'Ivoire and by Diouf (2006) on broiler meat in Senegal. Abba *et al.* (2017), others scientists in Tchad, obtained an average rate of 51.22 % on chicken meat. The high rate of resistance observed to Tetracycline in this study could explain its use in poultry farming, more specifically in the quail breeding. The investigation conducted during this study, showed that quail farmers use: antibiotics as prophylactic agentsto prevent the appearance of probable pathologies; for the treatment of disease cases and as a food additive or as growth factor for all types of poultry. These commonly used antibiotics belong to the cyclin family, which could explain the strong resistance to tetracycline observed in this work. However, a non negligible resistance to nalidixic acid (24 %) was also observed. This is confirmed in the work carried out on *Salmonella* strains by Bonny *et al.* (2014) on the raw chicken gizzard in Côte d'Ivoire (35.76 %), by Bouzidi (2013) on chickens in Algeria (63.15 %), by El Allaoui *et al.* (2017) on turkey in Morocco (37.1 %) and by Myskovâ and Karpîskovâ (2017) on poultry meat in the Czech Republic. All these observations could explain the probable use of Quinolones (nalidixic acid) during antibiotic therapy in poultry farming. The analysis of the different profiles observed in this study indicates that antibiotic families such as Quinolones, Sulfamides, Cyclins and β-lactams are the most involved in the antibiotic resistance of *Salmonella* strains isolated from quail viscerain Côte d'Ivoire. Indeed, according to Chaslus-Dancla (2003) the unreasonable use of

antibiotics generally, in intensive breeding leads to the appearance and selection of resistant bacteria. Also, the appearance of resistant profil involving these families of antibiotics would be the direct consequence of their uncontrolled use in the Ivorian poultry sector (Ouattara *et al.*, 2013).

Thus, the presence of *Salmonella* strains, resistant to antibiotics, isolated in quail viscera could be a problem of safety for this food.

Conclusion

This study on the prevalence of *Salmonella* strains in quail viscera revealed a high level of resistance to antibiotics such as tetracycline (100 %). Therefore, such an observation requires close to actors in the poultry sector, a control of their use to ensure the safety of these foods linked to human consumption.

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